

The University of Chicago

THE PHARMACODYNAMICS OF THE
DOMESTIC FOWL WITH RESPECT
TO ERGONOVINE AND
ERGOTAMINE

A DISSERTATION SUBMITTED TO THE
FACULTY OF THE DIVISION OF THE
BIOLOGICAL SCIENCES IN CANDIDACY
FOR THE DEGREE OF DOCTOR OF
PHILOSOPHY

DEPARTMENT OF PHARMACOLOGY

1939

By

MILTON JOHN VANDER BROOK

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THE PHARMACODYNAMICS OF THE DOMESTIC FOWL WITH
RESPECT TO ERGONOVINE AND ERGOTAMINE. By
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of the University of Chicago.

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INTRODUCTION.

THE relatively few pharmacological studies on the domestic fowl (*Gallus domesticus*) stand in marked contrast with the numerous physiological and nutritional studies which have been reported. Of historical interest are the experiments of Paracelsus [1658] on the hypnotic effects of ether on chickens. In more recent times Sharpe [1912-13] studied the secretion of urine in hens and found that they responded to the common diuretics in a manner similar to the more conventional laboratory animals. He experienced some difficulty in measuring avian blood-pressure. Hogben and Schlapp [1924], Hogben [1925], and Gaddum [1928] investigated the depressor response obtained with pituitary extracts in the fowl. Later it was shown by Morash and Gibbs [1929] that this action was a property of the oxytoxic fraction. Coon [1939] has proposed the use of this depressor response as a method of assaying the oxytoxic activity of posterior lobe extracts. Gibbs [1926, 1928, 1929] in his report on the action of various drugs on circulation in the chicken includes the information that ergotamine will give a prolonged rise in blood-pressure in this animal and in some instances an epinephrine reversal. He measured blood-pressure from the "popliteal" artery and rarely experienced difficulties.

Several workers have been concerned with the action of drugs on various isolated organs of the domestic fowl. These include Morash and Gibbs [1929] on the intestine, McKenney, Essex, and Mann [1932] on the oviduct, and Coon [1939] on the heart.

A few of the present bioassay methods use chickens. Among those which might be mentioned are chicks for antirachitic substances and gonadotropic and thyrotropic hormones, roosters for official ergot preparations, and capons for the male sex hormones.

The infrequency with which the fowl has been used is surprising when one considers the many advantages which it possesses over other laboratory animals. Conspicuous among these are its availability the

year round at moderate cost, its docility, its modest housing requirements, and finally its meagre emotional appeal to persons opposing vivisection. The most serious criticism which can be directed against the fowl is its distance from man on the phylogenetic scale, so that information obtained on it may not be assumed to apply to human biology. However, any animal experimentation is open to similar objection to a greater or lesser degree.

The present study of ergonovine and ergotamine on the fowl (White Leghorn) was undertaken both to add to the information concerning these alkaloids and to re-emphasise the practicability of utilising this somewhat neglected animal. No reports comparing the effects of these drugs on chickens have been found other than those dealing with toxic manifestations [Brown and Dale, 1935] and comb darkening [Brown and Dale, 1935; Thompson, 1935].

MATERIALS EMPLOYED.

The alkaloids employed were the maleate and tartrate of ergonovine, namely "Ergotrate" (Lilly) and "Basergin" (Sandoz), and the tartrate and methanesulphonate of ergotamine.¹ Since no differences in the biological response to the first two salts were found, the term "ergonovine" is used in this paper to refer to either the maleate or tartrate of the alkaloid. The term "ergotamine" is used instead of ergotamine tartrate for the sake of simplicity. Usually solutions were prepared from the solid material in quantities sufficient for two or three days' use. These were stored in a refrigerator. The solvent was 0.9 per cent. sodium chloride solution. In some instances sterile ampules of the alkaloids were employed. The ergotamine tartrate used in many of the experiments was a stock solution of "Gynergen." In no case could any variation in response be attributed to the type of solution in which the various alkaloids were administered. The pH of the ergonovine solutions was about 5.6, while that of the ergotamine tartrate solutions was about 3.4. Control injections included physiological saline, sodium tartrate, and tartaric acid.

BLOOD-PRESSURE STUDIES.

Thirty-five of the forty birds used in this part of the work were roosters, since they appeared to have a more uniform blood-pressure than hens. Sex, however, did not appear to be a determining factor with regard to the hemodynamics of ergonovine and ergotamine. Sodium phenobarbital, 200 mg. per kilogram intramuscularly, was used in all but one experiment. In this isolated instance a local anæsthetic (procaine) was used. No supplementary anæsthetic agent was required

¹ For the generous supply of these materials acknowledgment is made to the Eli Lilly Research Laboratory and to the Sandoz Chemical Works.

at any time. Artificial respiration when needed was administered by a Palmer pump. The blood-pressure was recorded by means of a mercury manometer connected to the ischiadic artery, and injections were made directly into the crural vein. A description of the technique employed is given by Coon [1939].

Ergonovine elicited a pressor response in thirty-seven of the chickens. A significant rise was obtained with amounts as low as 0.004 mg. (fig. 1),

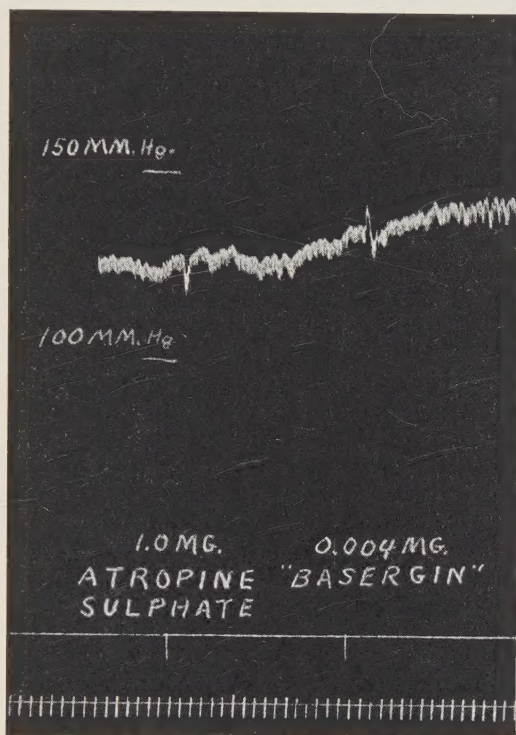


FIG. 1.—16.12.38. White Leghorn cock, wt. 2.18 kg. Phenobarbital-Na 200 mg./kg. I.M. Vagi cut. 38-V-R.

Blood-pressure response to a minimal dose of ergonovine.

and definite suggestions of a rise were obtained with only one-half the amount.¹ This quantity is considerably less than that reported necessary (0.1–1.0 mg.) to obtain blood-pressure changes in cats, dogs, or rabbits. When given to the fowl 0.1 mg. of ergonovine usually caused an elevation in pressure of some 30 mm. Equal doses of ergonovine and ergotamine produced approximately equal rises in blood-pressure, except in one or two cases in which the response to ergotamine was nearly twice that of ergonovine. This rise was quite prompt, occurring 10 or 15 seconds

¹ All quantities have been expressed in terms of total dose, not dose per kilogram.

following injection. In preparations in a state of shock the interval between injection and response was slightly longer, due presumably to sluggish circulation. In general the pressor effect of ergonovine was much more evanescent than that of ergotamine (fig. 2). This observation parallels that of the comb-darkening action of the ergot alkaloids,

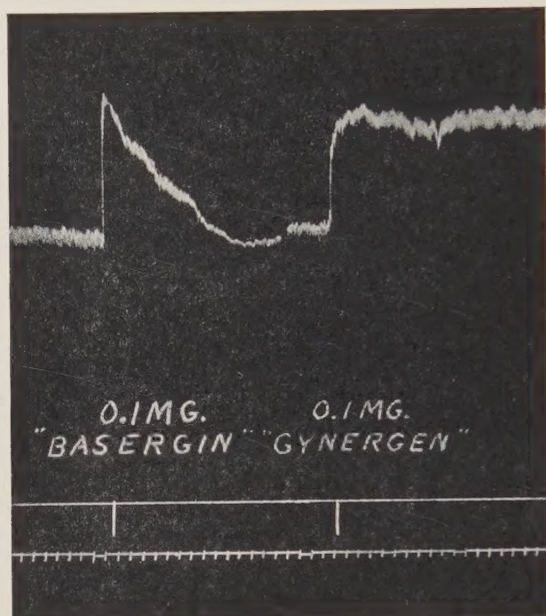


FIG. 2.—16.12.38. White Leghorn cock, wt. 2.0 kg. Phenobarbital-Na 200 mg./kg. I.M. 38-V-R.

A comparison of the pressor responses to ergonovine and ergotamine.

in which ergonovine has been reported to produce a prompt but more temporary darkening than ergotamine.

In three of the birds a fall in blood-pressure was observed with ergonovine although ergotamine elicited a rise (fig. 3). Another salt of ergonovine, the hydracrylate, and the free base itself in the form of "Ergometrine," also depressed the blood-pressure in these roosters. These three roosters did not appear abnormal and presented no peculiarities in their response to other drugs.

That the pressor effect obtained with ergonovine in the majority of cases was not an artifact of general anaesthesia was indicated by the results in the chicken when 0.1 per cent. procaine-hydrochloride infiltration anaesthesia was employed. Both alkaloids in the usual dosage range raised the pressure in a manner similar to that in fowls under phenobarbital anaesthesia. Vagotomy did not alter the pressor response in the chicken. Large doses of atropine (9 mg.) also left this response unchanged. The rise in pressure was slightly less in the decapitate

fowl, and a response was obtained even after pithing the cord. These experiments indicate that while most of the action is peripheral the central nervous system is responsible for at least part of it.

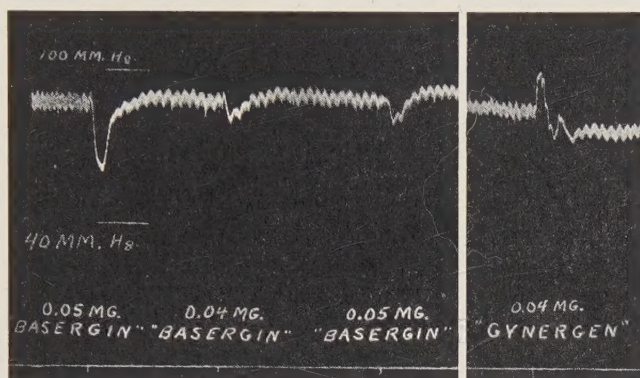


FIG. 3.—9.1.39. White Leghorn cock, wt. 1.75 kg. Phenobarbital-Na 200 mg./kg.
I.M. 44-V-R.

Fall in blood-pressure following ergonovine.

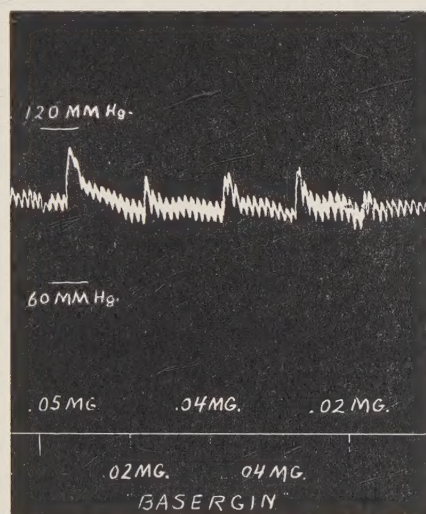


FIG. 4.—29.10.38. White Leghorn hen, wt. 1.9 kg. Phenobarbital-Na 200 mg./kg.
I.M. 5-V-R.

Pressor responses to varying doses of ergonovine.

With doses of ergonovine less than 0.1 mg. a fair correlation was found between the pressor response and the amount of the material injected (fig. 4). This fact in conjunction with the greater sensitivity of the chicken suggests its use as a means of checking the potency of solutions known to contain ergonovine.

Some variability in response to ergonovine was observed in this series other than the fall in blood-pressure noted in three cases. Although the pressure usually returned to its previous level quite promptly (3-6 minutes) following an injection of about 0.05 mg. of ergonovine, in some chickens the return was gradual, lasting from 8 to 12 minutes. In a few others the return was prompt, but was followed by a secondary rise which lasted about 5 or 6 minutes and was usually less than half the primary rise. In two instances injections of ergonovine following ergotamine elicited a weaker response than an equal dose given just before. On one occasion the injection of ergonovine following ergotamine produced a fall in blood-pressure, whereas this chicken gave pressor responses at other times during the experiment.

In the phenobarbitalised chicken a rise in blood-pressure followed doses of epinephrine-hydrochloride as small as 0.2 μ g., and the responses were fairly proportional to the amount of the drug given. In no case was it possible to obtain an epinephrine reversal following ergonovine, but an inhibition of the epinephrine response following ergonovine in amounts from 10 to 20 mg. was frequently observed. In some instances the response was nil, and in others it was definitely less than the epinephrine response preceding the ergonovine. After a recovery period of about 20 minutes the response to epinephrine was usually of about the same magnitude as before ergonovine. These observations parallel those of Brown and Dale [1935], who found that following large amounts of ergometrine (ergonovine) the pressor response of the spinal cat to epinephrine was much reduced but not reversed.

In the fowl epinephrine following ergotamine produced less of a rise in pressure than when given before, but in no instance was a reversal obtained. It is possible that the mild atropine-like action of phenobarbital was in part responsible for the absence of reversal following ergotamine [Herwick, Linegar, and Koppanyi, 1939]. The familiar cocaine sensitisation to epinephrine was readily demonstrated in the chicken.

Besides the circulatory changes in these anaesthetised fowls, ergonovine and ergotamine in doses as small as 0.01 mg. produced momentary respiratory depression. This was characterised by shallower breathing and a decrease in rate. Occasionally the depression was followed by a brief period of tachypnoea. The respiratory depression appears to be a primary effect of these drugs and not a result of reflexes caused by elevated blood-pressure, for the inhibition of respiration occurred even when ergonovine produced a depressor response. Quantities of the drugs sufficient to inhibit the response to epinephrine usually caused respiratory failure. In such instances artificial respiration was employed. The respiratory effects of ergonovine on the fowl are substantially the same as those reported for etherised cats [Davis, Adair, Chen, and

Swanson, 1935], in which doses of 5 to 30 mg. greatly suppressed the rate and amplitude and caused cyanosis and circulatory depression which were relieved by artificial respiration.

ELECTROCARDIOGRAPHIC STUDIES.

In order to demonstrate possible effects of ergonovine and ergotamine on the conducting mechanism of the heart, electrocardiograms were taken on phenobarbitalised chickens. Lead III was used in most cases. The electrodes were attached to the biceps region of each wing and to the tibial region of the left leg.

An injection of 0.05 mg. of ergonovine in one instance reduced the heart-rate from 188 to 162 beats per minute but did not otherwise alter the characteristics of the electrocardiogram. In another chicken the same amount changed the rate from 161 to 132 beats per minute. After decapitation the rate was only reduced from 172 to 164 beats per minute by 0.1 mg. of ergonovine. Following pithing of the cord this quantity increased the rate slightly. Ergotamine, 0.05 mg., decreased the heart-rate from 171 to 145 beats per minute. Following decapitation there was practically no change in rate with this dose. The electrocardiogram showed no changes other than that of rate. A large dose of ergonovine, 5 mg. per kg., slowed the heart and flattened the T-wave slightly. There were no other changes. Ergotamine in the same dose also slowed the heart, but in addition caused the T-wave to become diphasic. The P-R interval and QRS complex were not altered.

Pitocin, pitressin, epinephrine, neosynephrin, benzedrine, and ephedrine in amounts which produced moderate changes in blood-pressure had no effect on the electrocardiogram aside from minor changes in the heart-rate.

EXPERIMENTS ON ISOLATED ORGANS.

Heart.

Certain peculiarities in avian anatomy and physiology had to be considered in working with the isolated heart. In the first place the large sternum hinders one in getting at the heart. Additional delay is caused by two large branches which leave the aorta very close to the heart and which have to be ligated before perfusion can be started. The asphyxia resulting from these delays is often sufficient to produce irreversible damage. It was found that exposing the heart under either general anaesthesia with sodium-phenobarbital, or local anaesthesia with 5-10 c.c. of 0.1 per cent. procaine-hydrochloride, facilitated removal and produced a much more satisfactory preparation than could be obtained from chickens rendered unconscious by breaking the neck. The Ringer-Locke perfusion fluid was adapted to the physiology of the chicken by increasing its osmotic pressure (NaCl 0.95 per

cent.) and temperature (42° C.). The coronary flow in the isolated chicken heart was found to be two or three times that of the rabbit or cat and consequently exceeded the capacity of conventional drop recorders. An apparatus was designed which would handle this large volume and yet respond to fleeting changes in flow which are obscured by a bucket-type recorder. This device consisted of a stopcock which diverted a constant portion of the flow and permitted the remainder to fall as drops and actuate an electric recorder. These drops, together with the contractions of the heart, were recorded on a kymograph.

Hearts prepared in this manner and perfused at a pressure of 60 cm. beat vigorously from 1 to $4\frac{1}{2}$ hours, permitting the study of the effects of drugs added to the perfusion fluid just before it entered the coronaries. They did not, however, beat as long or as uniformly as cat and rabbit hearts which were used for comparison.

The most striking effect of ergonovine and ergotamine was found to be on the coronary flow. In every one of the 15 chicken hearts studied the first dose of either drug caused a marked reduction in flow. In the case of ergonovine quantities as small as 0.05 mg. produced a decrease of 25 to 35 per cent. Ergotamine appeared to be some ten times more potent. With larger amounts of either drug still greater diminution in coronary flow was obtained, as high as 43 per cent. with 0.1 mg. of ergonovine and 68 per cent. with 0.01 mg. of ergotamine. With ergonovine this decrease in flow lasted 10 to 15 minutes before a return to normal occurred, while with ergotamine recovery was slower and only rarely did the flow ever return to the initial level. In one heart an initial dose of 0.1 mg. of ergotamine reduced the coronary flow to 33 per cent. of its original rate, and although over 2 hours were allowed to elapse without any additional drugs being given, the flow was still only half of what it had been at the start. It should be noted in this connection that the ergotamine solution used was acid, and that the alkalinity of the perfusion fluid was sufficient to neutralise this and tended to precipitate out the ergotamine. The very persistent action of ergotamine is probably not due to a mechanical obstruction of the capillaries with precipitated material, since a degree of experimental air embolism sufficient to cause equivalent reduction in flow was accompanied by marked disturbances in the rhythm and amplitude of the heart such as were not observed with ergotamine. The low solubility of the alkaloid at a physiological pH may well contribute to its prolonged action by delaying its diffusion out of tissues once it has penetrated them. The possibility of the decreased coronary flow being due to a passive constriction of the capillaries by increased ventricular tone is excluded by the characteristic response to ergonovine and ergotamine obtained in hearts which had ceased to beat. The response of the coronary flow to these two drugs seemed to be independent of the type of anæsthetic used.

Only rarely were subsequent doses of these two alkaloids as effective as the initial one in reducing coronary flow. In the case of ergotamine this may be ascribed chiefly to the fact that the rate of flow was permanently reduced, and tended with each dose to approach a limit beyond which further slowing was impossible. With ergonovine, however, even though recovery was fairly rapid and complete, repeated administration gave evidence of tolerance. Thus an initial 0.05 mg. of ergonovine reduced the coronary flow from 37.2 to 27.0 c.c. per minute. One half-hour later the flow had returned to 41.2 c.c. per minute, and a second dose of 0.05 mg. of ergonovine only decreased it to 36.1 c.c. per minute. This is a 50 per cent. reduction in effectiveness.

There was definite evidence of cross tolerance in that when ergotamine was the first drug given to a heart; later doses of ergonovine were less effective in reducing the coronary flow than equivalent doses given to a fresh heart. Evidence of tolerance to ergotamine produced by ergonovine was suggestive but not as consistent. It is evident from a consideration of these results that a satisfactory comparison of the potency of these two alkaloids in reducing coronary flow is possible only by using a series of hearts and comparing the effects of the first drug given to each.

About half the hearts responded to ergotamine or ergonovine at some time during the experiment with a transitory increase in flow. This increase lasted about 10 seconds and was always immediately followed by the larger and more characteristic decrease. The increase was on the order of 20 per cent. It occurred most consistently when relatively large amounts of the drugs were used, *e.g.* 0.5 mg. or more of ergonovine. There was suggestive but not conclusive evidence that hearts removed from chickens under local or general anaesthesia were refractory to this action of ergonovine and ergotamine. The acceleration in coronary flow is not a part of a generalised sympathetic stimulation of the heart, as it can occur without any change in the rate or amplitude of the ventricular contractions.

The other actions of ergonovine and ergotamine on the isolated heart were inconspicuous in comparison with the effects on the coronary flow. Even with doses as large as 2.2 mg. of ergonovine and 0.1 mg. of ergotamine only minor unpredictable changes in the amplitude of contractions were obtained. Effects on frequency of contractions were equally irregular, with the exception of the tendency of an initial dose of ergonovine to accelerate hearts removed from phenobarbitalised chickens. It seems probable that the capricious action of these alkaloids is due to a mixture of sympathetic and parasympathetic effects [Gayet and Minz, 1936; Navratil, 1937], and that a mild parasympathetic depression resulting from phenobarbital [Linegar, Dille, and Koppanyi, 1936] may have permitted the sympathetic action to predominate.

Seven additional drugs (calcium chloride, epinephrine, ephedrine,

neosynephrin, tyramine, pitocin, and pitressin) were administered to isolated chicken hearts, but since their action on mammalian hearts is well known, and their behaviour on that of the chicken showed no unusual features, no detailed results will be presented. They served only to demonstrate that the avian heart responds characteristically to ordinary drugs. Similarly repeated doses of ergonovine and ergotamine were administered to 4 cat and 4 rabbit hearts. Since Donatelli [1938 *a*, 1938 *b*, 1938 *c*] has reported on the complex action of ergotamine and ergonovine on the mammalian heart, mention need be made here only of the effects on the coronaries, a phase apparently not included in his researches. It was found that the results obtained on cat and rabbit hearts paralleled in the main those already described for the fowl. Thus it was possible to demonstrate the prolonged decrease in flow, the initial transitory increase with large doses, and the development of tolerance. Experiments were not sufficiently numerous, however, to convincingly demonstrate such a complex relation as cross tolerance. Neither can any statement be made concerning the influence of preliminary anaesthesia since all these animals were stunned by a blow on the head.

The coronary constricting action of these ergot alkaloids has been ignored in the past. In the light of the present findings it would seem wise to give careful consideration before administering either of them to a patient suspected of having coronary disease.

Oviduct.

The oviducts of 7 hens were used in these experiments. Strips of the uterus, isthmus, and albumen-secreting portion were suspended in oxygenated Ringer-Locke solution and the response to ergonovine, ergotamine, ergotoxine, and epinephrine recorded.

With ergonovine the only consistent results were obtained on the uterine portion, which always showed an increase in tone to concentrations of this drug between 1 : 20,000 and 1 : 14,000. The response was frequently very slight, and with weaker concentrations no effects at all were obtained. The isthmus and albumen-secreting portion were inconsistent and feeble in their reaction to concentrations as high as 1 : 14,000 (fig. 5).

The response to ergotamine was completely erratic. Sometimes the tone and amplitude were increased; in other instances quite the reverse obtained. The drug seemed more powerful than ergonovine, however, for concentrations of 1 : 40,000 were rarely without effect in one direction or the other. Because of the report of McKenney, Essex, and Mann [1932] that all portions of the oviduct were consistently stimulated by ergotoxine at a concentration of approximately 1 : 600,000, this alkaloid was included in the present series of experiments although

there was little expectation that any real qualitative difference between it and ergotamine would be observed. It was found that ergotoxine-ethanesulphonate in concentrations of 1 : 4,000,000 to 1 : 80,000 were quite uniformly without effect on the oviduct, except in one instance in which a concentration of 1 : 80,000 caused a slight inhibition.

Epinephrine 1 : 2,000,000 to 1 : 500,000 elicited much more consistent responses than did the ergot alkaloids. All three portions of the oviduct

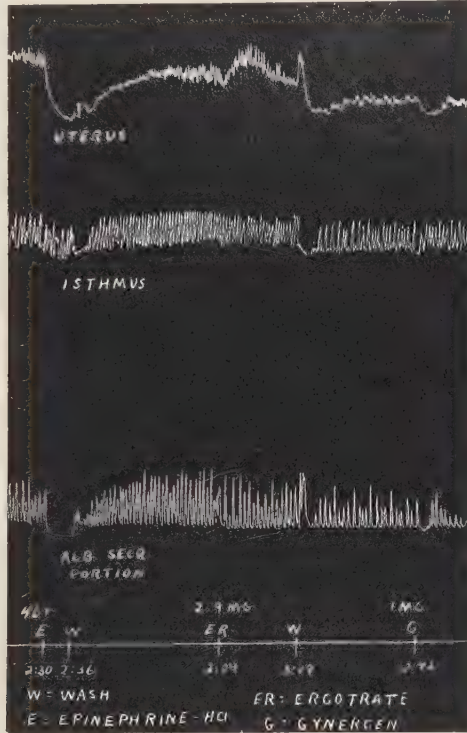


FIG. 5.—Response of the oviduct to epinephrine, ergonovine, and ergotamine.
Bath volume 40 c.c.

showed an inhibition of spontaneous contractions and a sudden loss of tone, except in the case of one strip of isthmus in which the tone was increased. These findings again disagree with those of McKenney, Essex, and Mann [1932], who state that the isthmus and albumen-secreting portion respond to epinephrine with a maximal contraction and only the uterus shows a relaxation.

It seems possible that the discrepancies between the findings reported here and those of McKenney, Essex, and Mann [1932] may be attributed to the fact that their birds were in active egg production, whereas such was not the case in the present series although the fowls appeared sexually mature.

Intestine.

Ergonovine caused a decrease in tone of isolated strips of gut but had little effect on the spontaneous contractions. The duodenum appeared most sensitive, being relaxed abruptly by a concentration of 1 : 400,000, and showing little tendency to recover even after washing. A concentration of 1 : 80,000 caused the jejunum to relax fairly abruptly, while this same concentration produced only slight relaxation in the ileum. The spontaneous contractions of the colon were the most vigorous. Ergonovine 1 : 400,000 caused a slight relaxation after the first dose, but had no effect even in much more concentrated solutions upon subsequent administration. On the colon of another chicken it required a concentration of 1 : 80,000 to elicit a slight relaxation.

Ergotamine 1 : 160,000 decreased the tone of the duodenum quite permanently. Its response was less abrupt than that of ergonovine. Concentration as high as 1 : 80,000 on the jejunum and ileum and 1 : 40,000 on the colon were ineffective.

SUMMARY.

1. The practicability of the chicken as a laboratory animal is reaffirmed.

2. The alkaloids ergonovine and ergotamine are about equally effective in their action upon the circulatory system of the chicken as judged by the magnitude of the blood-pressure response, but the effects of ergonovine are more transitory.

3. Much smaller doses of ergonovine are required to produce pressor responses in chickens than in cats or in other laboratory animals.

4. In doses of about 10–20 mg. ergonovine and ergotamine are effective in inhibiting but not reversing the blood-pressure response to epinephrine in the chicken.

5. After moderate doses of ergonovine and ergotamine electrocardiograms show only a slowing of the heart. With excessive doses minor changes in the T-wave are observed.

6. In experiments on the perfused hearts of the fowl, cat, and rabbit small amounts of ergonovine and ergotamine give a striking reduction in the coronary flow. Ergotamine is some ten times more potent than ergonovine in this respect and also more persistent.

7. The response of the oviduct to these alkaloids is irregular except in the case of the uterine portion, which is always stimulated by ergonovine. Epinephrine inhibits all segments.

8. Studies on the isolated chicken intestine show a reduction in tone from ergonovine and ergotamine, the former being more effective. The response to both substances decreases from duodenum to colon.

It is a pleasure to acknowledge the helpful guidance of Professor E. M. K. Geiling throughout this investigation. A portion of the

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